J. Physiol. (1957) 135, 339–349

# THE EFFECT OF INHIBITION OF AMINE OXIDASE *IN VIVO* ON ADMINISTERED ADRENALINE, NORADRENALINE, TYRAMINE AND SEROTONIN

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(*Received* 30 *August* 1956)

Zeller & Barsky (1952) showed that 0·1 mM/kg of 1-*iso*nicotinyl-2-*iso*propyl hydrazine (IIH or Marsilid) injected subcutaneously into rats completely inhibited amine oxidase activity in homogenates of liver taken from animals killed after two hours. Larger amounts (0·18 mM/kg) reduced activity in guinea-pig liver to 6 % of normal. Zeller, Barsky & Berman (1955) state that 0·4 mM/kg completely inhibited liver homogenate activity in the dog but that brain amine oxidase was only reduced by 73 % in the same period of two hours. It was effective in the rat and guinea-pig. This inhibition of monoamine oxidase *in vivo* is said to occur in cats also. The compound choline-*p*-tolyl ether bromide (TM 6) has been shown to be an effective inhibitor of guinea-pig liver amine oxidase activity *in vitro* (Brown & Hey, 1956) and to modify the excretion products of tyramine in rats and mice (Schayer, 1953) and of tryptamine in mice (Schayer, Wu, Smiley & Kobayashi, 1954) in a manner which is presumed to indicate such activity.

The role of amine oxidase in the biological inactivation of adrenaline and noradrenaline was reviewed by Blaschko (1952). Bacq (1949) considered this enzyme to be of little significance *in vivo*, and recently von Euler & Hellner-Björkman (1955) and von Euler & Zetterström (1955) have reached the same conclusion on evidence from animal experiments and human excretion studies. On the other hand, Schayer (1951), working with isotopically labelled adrenaline, concluded that amine oxidase was of major importance in its catabolism. Sjoerdsma, Smith, Stevenson & Udenfriend (1955) have shown that prior administration of IIH to rats, rabbits or dogs inhibits the activity of the liver amine oxidase on 5-HT as substrate when measured manometrically. An attempt has accordingly been made to gain information by using amounts of

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inhibitor which were demonstrated to be effective in the species used for the times required and examining the effect on the excretion of infused adrenaline, noradrenaline and serotonin (5-HT) and on inactivation of infused adrenaline, noradrenaline and tyramine in the tissues.

## METHODS

### *Inhibition of amine oxidase*

*In vitro.* An acetone-dried preparation of guinea-pig liver was made by homogenizing livers of six guinea-pigs in 5 vol. of dry ice-cold acetone for 2 min, filtering under vacuum, resuspending the powder in 5 vol. cold acetone and repeating the procedure three times. The powder was dried on paper during the night, ground and sieved at 60 mesh (Blaschko, personal communication). As needed it was washed twice with 0·0667 M-phosphate buffer at pH 7·4 and suspended at a concentration of 100 mg/ml. The optimal activity of this preparation was found to occur at pH 7·2–7·6 and the velocity constants for $O_2$ uptake by enzyme-substrate mixture remained unchanged over a period of a year for tyramine, adrenaline and noradrenaline as substrates. Activity was measured by conventional manometry at 37° C in $O_2$, final volume per flask 2·4 ml., with tyramine as substrate in concentrations of $5{\cdot}2$–$2{\cdot}08 \times 10^{-3}$ M; with and without addition to the enzyme of IIH and TM6 in concentrations of $10^{-3}$–$10^{-5}$ M for 30 min before final mixing.

*In vivo.* 1·0 ml. of fresh liver homogenate, obtained from twelve guinea-pigs and from six cats anaesthetized with 40 mg/kg pentobarbitone sodium, was used as enzyme source, and duplicate activity was determined manometrically as described above with $3{\cdot}125 \times 10^{-3}$ M-tyramine as substrate. The livers were homogenized with twice their wt. in ml. of cold buffer. Results were expressed as $\mu$l. $O_2$ consumed/g wet liver/hr, determined 30 min after adding substrate. The experiments were repeated twice on groups of three guinea-pigs or individually in two cats killed at varying periods of time after intravenous (I.V.) or intraperitoneal (I.P.) injection of 10 mg/kg IIH or TM6. The presence of active amine oxidase in rat, cat and guinea-pig kidney slices was demonstrated histochemically by the technique of Blaschko & Hellmann (1953) and used to determine the activity of IIH and TM6 *in vivo.*

### *Inhibitors and inactivation of circulating amines*

Brief infusions of L-adrenaline (1·5 $\mu$g/kg/min), L-noradrenaline (0·75 $\mu$g/kg/min) with ascorbic acid $10^{-6}$, or tyramine (40–80 $\mu$g/kg/min) were given to cats anaesthetized with 40 mg/kg pentobarbitone sodium. The amine was administered by retrograde infusion into a branch of the anterior mesenteric artery until a steady rise in carotid pressure resulted. The infusion was then immediately switched to a portal vein cannula and after re-stabilization of the pressure to a cannula in the jugular vein. When responses were constant and repeatable IIH was injected intravenously in a dose of 20 mg/kg and the tests repeated regularly over a period of 2–3 hr. The method is a modification of Celander & Mellander (1955).

### *Inhibitors and excretion of amines*

Cats were anaesthetized with pentobarbitone sodium 40 mg/kg I.P. and the carotid pressure was recorded. Continuous I.V. infusions of approx. 4 $\mu$g/kg/min L-adrenaline or 2 $\mu$g/kg/min L-noradrenaline stabilized with ascorbic acid $10^{-6}$ were given for 5 hr; the precise amount was adjusted to give a suitable rise in pressure and was measured. Urine was collected from a cannula in the urethra into a flask containing 0·5 ml. conc. HCl, surrounded by ice, during and for 1 hr after the infusion, and amines were extracted by the following modification of the methods of Pekkarinen & Pitkänen (1955) which are based on the method of Lund (1950) for blood. The urine was brought to pH 8·5 with 5 N-NaOH and phosphates and sulphates were precipitated by the addition of 0·2 N-barium chloride, and centrifuged. Activated aluminium oxide (chromatographic) 4 g/100 ml. was added to the filtered supernatant urine, the pH re-adjusted to 8·5, and the mixture poured into a 12 mm glass column constricted and plugged with cotton-wool at the exit. 500 ml. distilled water was passed through the column followed quickly by 10 ml. of 0·2 M-sodium acetate. The adsorbent was then eluted with 20 ml. of 0·2 N-acetic acid followed by 20 ml. distilled water. The total adrenaline or noradrenaline content of the original urine was contained in the mixed eluates and was determined as follows. For adrenaline a 10 ml. aliquot of eluate was brought to pH 3 by addition of N-HCl, 20 mg $MnO_2$ was added and it was shaken for exactly 1 min, then quickly filtered. To the filtrate was added 1 ml. of 5 N-NaOH containing 0·2% ascorbic acid. The resulting fluorescence was measured immediately at 500 m$\mu$. Blanks prepared without ascorbic acid were read 30 min after addition of the alkali and the values subtracted from the amine content as read from standard calibration curves. Conjugated amine was estimated after hydrolysis by HCl and boiling for 20 min or treatment for 2 hr with $\beta$-glucuronidase and aryl sulphatase at pH 5·5 and 37° C contained in 100 mg of an acetone-dried preparation of limpet viscera (Dodgson & Spencer, 1953). Eight cats were used for each control estimation of excretion of L-adrenaline and L-noradrenaline, to determine the percentage of the total amine infused which was recoverable and the time relations of excretion. Thereafter two groups of seven cats were used each with L-adrenaline and L-noradrenaline after treatment with 20 mg/kg IIH I.P. 2 hr before and 1 hr after infusion, and 20 mg/kg TM6 I.P. 1 hr before and 1 hr after infusion. In addition five cats were infused for 3 hr with 5-HT 21–25 $\mu$g/kg/min and another five cats similarly treated 2 hr after IIH 20 mg/kg I.P. The urine was collected into acid surrounded by ice during and for 3 hr after the infusion and stored at −10° C until the content of 5-OH-indoleacetic acid (5-HIAA) was estimated according to Udenfriend, Titus & Weissbach (1955).

## RESULTS

### *Inhibition of amine oxidase*

*In vitro.* Table 1 shows the mean results obtained with IIH and TM6 on three preparations of acetone-dried guinea-pig liver. The number of trials made with each concentration of inhibitor is stated in brackets after the figure indicating the percentage inhibition of activity as determined from the controls. It will be seen that TM6 and IIH are of comparable potency when measured in this way with tyramine as substrate. Adrenaline and noradrenaline as substrates gave similar results.

*In vivo.* Since both TM6 and IIH had been found to act as inhibitors of amine oxidase when added *in vitro*, it was decided to examine their effectiveness when injected into the living animal. Table 2 shows the effect of prior intraperitoneal injection of IIH in a dosage of 10 mg/kg to cats and guinea-pigs. Peak activity occurs after 2 hr but there remains a 50% reduction in the activity of excised liver from guinea-pigs after 12 hr as measured in the Warburg respirometer with tyramine as substrate. The restoration of amine oxidase activity in the cat liver was not followed beyond 2 hr for lack of materials. In contrast to the previous finding that TM6 and IIH are of comparable potencies when added *in vitro* to the enzyme preparation, it may be seen that TM6 is practically inactive when injected in this dose in these species. Intravenous injection of the inhibitors did not alter the findings, nor the use of adrenaline or noradrenaline as substrate.

When thick frozen sections of kidney or liver are incubated with tryptamine, the endogenous amine oxidase causes production of a brown pigment. Prior incubation with various concentrations of inhibitor for different times may modify production of pigment, or one kidney may be removed from an anaesthetized animal and used as control on the pigment formation in slices from the other kidney excised after dosing the animal for 2 hr with 10 mg/kg of IIH or TM6. Incubation after adding substrate was always carried out for 1 hr. Compound TM6 in final concentrations of $10^{-3}$, pre-incubated with kidney slices for 45 min, failed to inhibit pigment formation in cat or guinea-pig, and only partially inhibited it in rats; $10^{-4}$ had no effect. Injection of TM6 10 mg/kg by the intravenous or intraperitoneal routes into cats, rats or guinea-pigs failed to produce any difference in subsequent pigmentation of slices from

TABLE 1. The percentage reduction of oxygen consumption by an acetone-dried preparation of guinea-pig liver (100 mg/ml.) and tyramine ($5\cdot208 \times 10^{-3}$ M) used as a substrate after 30 min treatment with IIH or TM6 in concentrations of $10^{-3}$–$10^{-5}$. The numbers in brackets indicate the number of experiments made in each case

| Inhibitor and concentration (M) | Inhibition (%) |
|---|---|
| IIH $10^{-3}$ | 100 (5) |
| $10^{-4}$ | 40·3 (4) |
| $10^{-5}$ | 11·3 (4) |
| TM6 $10^{-3}$ | 100 (3) |
| $10^{-4}$ | 39·3 (7) |
| $10^{-5}$ | 21·7 (4) |

TABLE 2. Mean of two estimates of the percentage reduction of amine oxidase activity in fresh liver homogenate from groups of three guinea-pigs or two individual cats, measured at varying intervals of time after intraperitoneal injection of IIH or TM6 in doses of 10 mg/kg. Control values were obtained from twelve guinea-pigs and six cats

| Inhibitor | Species | 1 hr | 2 hr | 6 hr | 12 hr |
|---|---|---|---|---|---|
| IIH | Cat | 75·4 | 100 | — | — |
| IIH | Guinea-pig | 88·6 | 100 | 83·3 | 51·9 |
| TM6 | Cat | 0 | 0 | — | — |
| TM6 | Guinea-pig | 2·5 | 0 | — | — |

the controls or treated organs. In contrast, IIH completely inhibited pigment formation when incubated with tissue slices at a concentration of $10^{-4}$ and had a considerable effect at $10^{-5}$ in all three species. Time is necessary for the inhibition to develop, a concentration of IIH of $10^{-3}$ being ineffective if added with the substrate to guinea-pig kidney slices but fully effective if added 10 min before the substrate; $10^{-4}$ requires 45 min and $10^{-5}$ 2 hr. Intravenous or intraperitoneal injection of 10 mg/kg IIH in cats, rats or guinea-pigs inhibits pigment formation in kidney slices taken 2 hr later. Of the two compounds, IIH is the more effective *in vivo* in non-toxic doses and was chiefly relied upon as an inhibitor of amine oxidase in further experiments.

*Inactivation of circulating amines*

Infusions of physiological amounts of adrenaline (1·5$\mu$g/kg/min) or noradrenaline (0·75$\mu$g/kg/min), or of larger amounts of tyramine (40–80$\mu$g/kg/min), into the anterior mesenteric artery, have little effect on the systemic blood pressure, indicating that most of the amine is metabolized or stored in its passage through the tissues supplied by the artery. The same amounts given via the hepatic portal circulation are more effective though much inactivation does occur in the liver. Jugular or other systemic venous infusion produces the most marked pressor response. These graded responses to the same rate and dose of infusion are shown in Fig. 1 for L-adrenaline and tyramine. If the acti-

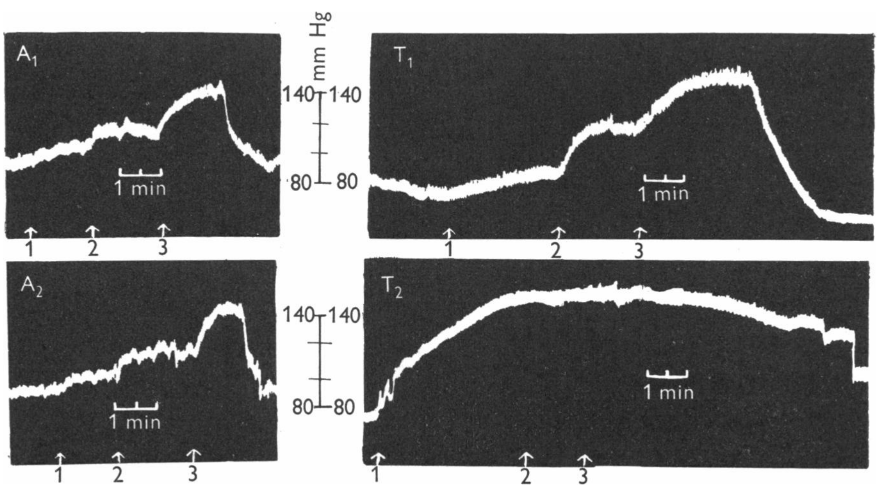


Fig. 1. Carotid blood pressure of two cats, 2·3 and 2·7 kg weight, pentobarbitone sodium 40 mg/kg I.P. ↑, brief infusions; 1, intra-arterial; 2, intraportal; 3, intrajugular; A, of L-adrenaline 1·5$\mu$g/kg/min and T, of tyramine 40$\mu$g/kg/min: between $A_1$ and $A_2$ and between $T_1$ and $T_2$, IIH 20 mg/kg acting for 2 hr. Time, 1 min.

vity of amine oxidase is inhibited by intravenous administration of 20 mg/kg IIH and the brief infusions carried on in series until in each case a plateau of pressor response is reached, it is found that in the case of infusions of L-adrenaline and L-noradrenaline the inhibitor does not alter the pattern of responses over a period of two or three hours. When tyramine is infused, the response to intra-arterial and intrahepatic portal infusion increases and after 2 hr there is no difference in response by the three routes of administration. The contrast between the effect of IIH on the responses to adrenaline and tyramine is shown in Fig. 1. Inhibition of amine oxidase in the cat, therefore, does not modify pressor responses to adrenaline and noradrenaline but markedly affects the response to tyramine.

*Inhibitors and excretion of amines*

In control experiments different amounts of amine were added to varying volumes of cat urine, subjected to the extraction process and estimated fluorimetrically. Recovery of L-adrenaline in ten trials was 96–100·3% (average 98·4) and of L-noradrenaline 92–115% (average 98·3). No correction was applied to the subsequent experimental findings. No detectable amounts of free or conjugated amine were found in the urine produced by anaesthetized cats infused with saline. But when amine was added to the infusion small amounts were recovered, as shown in Table 3; the average amount was 2·54% for adrenaline and 3·7% for noradrenaline. Variation in the rate of infusion within the limits imposed by the cardiovascular response has no effect on the percentage excretion of free amine. In only two of the urines from sixteen cats investigated was a minute amount of conjugated amine found (0·4% of L-noradrenaline and 0·5% of L-adrenaline). The bulk of these amines which appears in free form is excreted within the period of the infusion and 1 hr thereafter. This fact was established by collecting urine for various prolonged periods after the infusions ceased and estimating the amine in the samples. 5-HT may be infused in larger amounts (25$\mu$g/kg/min) for shorter periods and produces a fall in carotid blood pressure. The metabolic state may be determined by estimation of 5-HIAA in the urine; some 52% appeared thus within 3 hr. Intravenous injection of 20 mg/kg of IIH or TM6 did not cause an excretion of free adrenaline or noradrenaline in the urine of cats infused with saline. After IIH the percentage of infused adrenaline excreted as free adrenaline rose to 5·2 and of infused L-noradrenaline excreted as free amine to 5·4, a statistically significant rise, whereas the excretion of infused 5-HT as 5-HIAA fell from 52 to 18% as shown in Table 3. After TM6 20 mg/kg there was no significant alteration in the output of free amine. This compound was not used in the experiments with excretion of 5-HIAA after infusion of 5-HT. The use of IIH or TM6 had no effect on excretion of conjugated amine: this was only detected to the extent of 0·4 and 0·7% in the urine of two cats infused with adrenaline and given TM6, and not at all after IIH or noradrenaline in the treated series.

Infusion of L-adrenaline at a rate of 4$\mu$g/kg/min causes a rise in carotid pressure which is maintained for about 15 min and slowly returns to normal after about 1 hr, despite maintenance of the infusion at a steady rate, and may fall below the initial level. With noradrenaline in half this amount the rise is quicker and greater, the plateau is maintained for about an hour at a level less than that which was first attained and the return to, but not below, the initial level takes about 4 hr. With both amines the pattern of response may then be repeated by increasing the rate of infusion.

TABLE 3. Recovery (%) of free urinary L-adrenaline and L-noradrenaline and of 5-HIAA in cats anaesthetized with pentobarbitone sodium 40 mg/kg and infused with L-adrenaline, L-noradrenaline or serotonin; and the effect of 20 mg/kg of IIH and TM6

| | Adrenaline | | Noradrenaline | | Serotonin | |
|---|---|---|---|---|---|---|
| | Rate of infusion ($\mu$g/kg/min) | Recovery of free amine (%) | Rate of infusion ($\mu$g/kg/min) | Recovery of free amine (%) | Rate of infusion ($\mu$g/kg/min) | Recovery of 5-HIAA (%) |
| Controls | | | | | | |
| | 4·1 | 1·9 | 2·2 | 3·1 | 25·1 | 42·5 |
| | 1·4 | 1·6 | 1·5 | 4·3 | 21·9 | 46·7 |
| | 7·0 | 3·1 | 2·4 | 3·2 | 24·1 | 57·7 |
| | 6·4 | 2·2 | 1·25 | 4·1 | 23·1 | 61·2 |
| | 4·5 | 2·9 | 2·7 | 3·8 | 23·5 | 54·3 |
| | 8·5 | 2·3 | 2·0 | 3·7 | — | — |
| | 3·4 | 4·0 | 1·8 | 3·4 | — | — |
| | 4·0 | 2·3 | 2·0 | 3·9 | — | — |
| Mean ± S.E. | | 2·54 ± 0·27 | | 3·7 ± 0·15 | | 52·48 ± 3·79 |
| IIH 20 mg/kg | | | | | | |
| | 4·9 | 4·6 | 2·1 | 5·8 | 23·1 | 32·5 |
| | 4·2 | 4·0 | 2·7 | 5·7 | 23·7 | 16·1 |
| | 4·5 | 5·4 | 2·2 | 4·4 | 26·2 | 12·3 |
| | 4·0 | 4·9 | 2·2 | 6·2 | 22·7 | 11·7 |
| | 5·7 | 6·6 | 1·8 | 3·8 | 24·1 | 17·4 |
| | 4·0 | 5·7 | 2·4 | 5·9 | — | — |
| | 4·2 | 5·3 | 1·9 | 6·1 | — | — |
| | — | — | — | — | — | — |
| Mean ± S.E. | | 5·2 ± 0·31<br>$t$ = 6·45<br>$P$ < 0·001 | | 5·4 ± 0·35<br>$t$ = 4·61<br>$P$ < 0·001 | | 18·0 ± 4·23<br>$t$ = 1·16<br>$P$ < 0·001 |
| TM6 20 mg/kg | | | | | | |
| | 5·9 | 4·4 | 1·7 | 3·1 | — | — |
| | 3·7 | 4·1 | 2·0 | 2·0 | — | — |
| | 4·2 | 2·3 | 2·2 | 1·8 | — | — |
| | 5·0 | 1·9 | 2·2 | 1·7 | — | — |
| | 4·0 | 3·5 | 1·7 | 2·3 | — | — |
| | 4·0 | 2·7 | 2·0 | 4·7 | — | — |
| | 4·5 | 2·1 | 2·0 | 4·1 | — | — |
| | — | — | — | — | — | — |
| Mean ± S.E. | | 3·0 ± 0·38<br>$t$ = 1·04<br>0·4 > $P$ > 0·3 | | 2·6 ± 0·35<br>$t$ = 2·11<br>0·1 > $P$ > 0·05 | | |

## DISCUSSION

The preliminary experiments on the efficacy of IIH as an inhibitor of amine oxidase activity confirm the findings of Zeller & Barsky (1952) and Zeller *et al.* (1955). There appears to be a species difference between rats and guinea-pigs in the rate of recovery of amine oxidase, the latter workers having shown that after 9 mg/kg subcutaneously activity in rat liver is still only 50% after 3 days, whereas we found the same degree of recovery in guinea-pigs with a similar dose after 12 hr. The finding of Brown & Hey (1956), that choline-*p*-tolyl ether bromide is an inhibitor of amine oxidase activity when added *in vitro* to liver slices, is confirmed by the more conventional method of addition to homogenate in the respirometer vessel but not when it is added in a concentration of $10^{-3}$ for 45 min to slices and substrate for formation of pigment. When injected *in vivo* in the doses used (10 mg/kg) this compound was found to be ineffective as an inhibitor by two methods—activity of liver homogenate in the respirometer and pigmentation of kidney and liver slices—in cat, rat and guinea-pig. This finding suggests that the inhibition of amine oxidase exerted by TM6 may be readily reversible in these species, whereas that exerted by IIH is not: this is illustrated in Table 2. Schayer *et al.* (1954) gave 50–200 mg/kg subcutaneously to mice and 2–10 mg/kg subcutaneously to rats for 30 min and obtained alterations in the pattern of metabolites excreted after administration of tryptamine labelled with isotopic carbon. They interpret this as evidence of the inhibition of amine oxidase by TM6 and the significant role played by the enzyme in catabolism of monoamines. The higher doses given to mice approach the toxic level. Only the upper level of dosage appears to be effective (Schayer, 1953), which implies a marked species difference between the mouse and the rat in the effectiveness of this compound. The anomalous feature appears to us to be that in the many reports by Schayer and his colleagues of careful and exhaustive studies with isotopically labelled sympathomimetic amines, TM6 appears to have given similar results to IIH on the few occasions on which it was used in mice and rats. Nevertheless, it seems to us desirable that the activity of an inhibitor should be checked by as many means as possible, and in several species, before its action is relied upon in interpreting important findings. On these grounds we consider that work with TM6 is of less value than work with IIH.

A number of workers have shown that inhibition of amine oxidase by IIH does not affect the actions of adrenaline on various test organs but that those of tyramine are potentiated (Griesemer, Barsky, Dragstedt, Wells & Zeller, 1953; Furchgott, Weinstein, Huebl, Bozorgmehri & Mensendiek, 1955). This finding has been confirmed and extended to include noradrenaline which behaves like adrenaline. If the actions of one substance (tyramine) known to be a substrate of a particular enzyme (amine oxidase) *in vitro* is potentiated by the inhibition of that enzyme *in vivo* (by IIH) then the actions of other substrates (adrenaline and noradrenaline) should also be potentiated. That this is found not to be so in the experiment of Celander & Mellander (1955) indicates that the inactivation of these two amines in the cat is either but little dependent on amine oxidase activity or that some other mechanism can take over disposal of these amines but not of tyramine when this enzyme is inhibited. The difference in response to adrenaline and noradrenaline and to tyramine after IIH has only been reported and illustrated for one technique but was confirmed on other preparations.

Unfortunately no method could be devised for estimating tyramine excreted in urine, and therefore 5-HT estimated as its end product 5-HIAA was substituted in the excretion studies. The recovery of 52% of the amount infused is higher than the amounts reported by Erspamer (1954), though the intravenous route increases the output and there is marked species difference, the cat not being mentioned in this part of his review. The output of free adrenaline and noradrenaline agrees with the figures provided by von Euler & Zetterström (1955) in human beings. The absence of conjugation products of these two amines after exogenous administration confirms the findings of Schayer (1951) and von Euler & Zetterström (1955) among others, although it has been shown to be a route of excretion for endogenous adrenaline and noradrenaline in humans (von Euler, Hellner-Björkman & Orwen, 1955). It may be that the technique of hydrolysis used by us was not powerful enough, especially if an amine glucuronide was present. The limpet enzyme may not act on conjugates of adrenaline and noradrenaline, but it seems improbable that conjugation is of any significance in the catabolism of injected amines or that inhibition of amine oxidase causes this route to become important. The excretion studies demonstrated the inactivity of TM6 in modifying the excretion of adrenaline and noradrenaline in the cat in the doses given, which is in agreement with the findings on amine oxidase activity in liver and kidney but differs from Schayer's result in the rat.

In conclusion we have demonstrated the superiority of IIH over TM6 as an inhibitor of amine oxidase *in vivo*. Using effective doses of IIH we have found that the pressor response to adrenaline and noradrenaline infused by several routes is not affected by inhibition of the enzyme, while that of tyramine is. Balzer & Holtz (1956) also found that IIH increases responses to tyramine injected intravenously. Further, the excretion of infused adrenaline and noradrenaline in urine as free amine is doubled (Corne, 1956) but the amounts so affected are very small and 95% remains unaccounted for, whereas the excretion of 5-HT metabolites is profoundly affected. We suggest, therefore, that amine oxidase only plays a minor part in the disposal of circulating adrenaline and noradrenaline.

## SUMMARY

1. Choline-*p*-tolyl ether bromide (TM6) and 1-*iso*nicotinyl-2-*iso*propyl hydrazine (IIH) are effective inhibitors of amine oxidase in liver when added to it *in vitro*.

2. IIH in a dose of 10–20 mg/kg is completely effective two hours after injection in inhibiting liver and kidney amine oxidase in the rat, cat and guinea-pig as examined by respirometry of homogenate and pigmentation of slices.

3. TM6 in the same amounts is not effective in these species when examined by these techniques.

4. Inhibition of amine oxidase in the cat with IIH alters the pattern of pressor responses to tyramine infused intra-arterially, intraportally and intravenously but not the responses to adrenaline or noradrenaline.

5. Inhibition of amine oxidase in the cat with IIH raises the urinary excretion of free adrenaline from 2·5 to 5·2% and of noradrenaline from 3·7 to 5·4%. No conjugation was observed.

6. The excretion of 5-hydroxy-indoleacetic acid resulting from infusion of 5-HT in cats fell from 52 to 18% after similar treatment with IIH.

7. These findings are interpreted as indicating a more significant role for amine oxidase in the catabolism of such amines as 5-HT and tyramine than adrenaline and noradrenaline when they are administered into the circulation of cats.

This work was performed while one of us (S.J.C) was in receipt of an Educational Grant from the Pharmaceutical Society of Great Britain.

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